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Cavi di L.,19/8/49

Dear Lederberg,

Thank you for your letter of the 27th July. Your findings were of extreme interest to me, and I am very glad to hear that you have been able to give a demonstration that Hfr is actually a sexually more potent strain,rather than a less intersting strain owing its properties in recombination experiments to some physiological activities. I had not been able to show that Hfr gives recombination also when recombination is not necessary, though I had employed a different tehhnique I tried twice to grow together in broth Hfr/and W 583,after 24 h incubation I plated them on EMB lactose plates coated with phages T_1 and T_6 ; results were always obscured by a high mutation rate to resistance in the controls,and I never thought of testing colonies for recombination ,as you did. Your results are,I think,also of great interest in showing thatrecombination occurs also if it is not needed;and they are very encouraging for my plans of following with micromanipulations what happens during and after fusion.

If you go on with your experiments of copulation on complete medium,you may be interested to know that Hfr is rather slow in "copulation". I have followed the growth of colonies of prototrophs obtained with Hfr or with 58-161 ,in comparison of controls : 58-161 has an average lag of about 6 hours ,while with Hfr the delay is more than double. There is a great variation ,in both cases,between the lag of single prototrophs.

As to the differences between your data and mine, I think two points,which may not be identical in your and mine technique, may be of importance. When I mean "moist" plates,I actually mean plates which have been prepared shortly before use. No more than 0.05 ml. can be plated on these plates,to obtain clear cut colonies. Results are,however,usually good, even if the plates are used as soon as agar is solidified. Moreover, I think salts have great importance. I have not yet done any systematic research on the subject,but have many hints for it,and I think it may be worth while planning a research about it. I prepare a solution of salts in 10 times concentration,which is then added in the plate before pouring a test tube containaing a measured quantity of ghucose and agar. Preparing a 10 x

concentrated salt solution, some precipitate is formed even when salts are added one after the other, dissolving them carefully each time. This precipitate is removed by filtration, but precipitation continues slowly in the concentrated solution. Once that I used a salt solution in which a larger precipitate than usual had occurred, all recombinations were extremely low, and returned normal only when I changed the salt solution. I am using the same salts, in the same proportions you suggest in your 1947 paper.

I am quoting all these rather poor data at length, to suggest possibilities, because it is quite worth while testing possible origins of the discrepancies you find.

As to the efficiency of the experiment with "asymmetrical" input of cells of either strain, with 58-161, I have done it only in the absence of vitamin B_1, and I did not notice any high efficiency.

I am very interested to know if you have been able to recover the Hfr behaviour in recombinants. I tested only 8 recombinants for this behaviour, and none of them was Hfr; two were, however, rather higher than 58-161, giving nearly 10x more prototrophs. As to the oppositional character of the Hfr effect, I think to remember correctly saying that Ar- x B_1- gave about 10^{-4} prototrophs. However, I could not localize, due to the difficulties of interpreting crossover data with Hfr mentioned in the Cambridge paper, the two genes, and they may be far apart or close together; it may well be worth repeating the experiment with other substrains of Hfr.

Concerning strain 123, experiments of outcrossing K 12 had been started, after finding Hfr, with a view to test the possibility of mating types; however, Hfr is no more effective than 58-161 when crossed to 123. The result itself seemed to me however, intersting enough, though I have been long time thinking about the persiste of parental characters, and its meaning. There remains the possibility, which you mention, that syntrophism, Lactose instability of 123 (I have no data about it), lysogenicity of K12 concur to give an illusion of recombination. However, crossing 123 $V^R_{1,5}$ with 58-161 one gets approximately the reverse proportions of sensitives and resistants. The fermentation characters which do not recombine at all are th more closely linked with M-; I could never use other biochemical markers on the K12 side, because W583 x 123 gives extremely tiny colonies, tinier than 123 itself,

of very slow growth, and therefore ~~xxx~~ difficult to test.

I was very interested to hear about your ~~xxxxxxxxxx~~ experiments with Salmonellae. My experiments with other coli strains had been started, curiously enough, with the idea of using drug resistances, as you did. However, I had only streptomycin resistance in mind, after Monod's suggestion; Ny^R did not work, for this purpose, there being no concentration of Ny which kills all sensitives and allows survival of all resistants. So I started using streptomycin-sensitiveness on the side of the new coli strains (we used, in the first instance, all those giving growth on minimal medium) and biochemical deficiencies in K 12 streptomycin resistant strains, on a minimal medium with streptomycin. The trouble was that, to avoid killing all bacteria at once, we had to add streptomycin in a fourth layer, some hours later, and we never had any satisfactory results. Have you good experience of similar techniques?

I shall certainly send you 123, as soon as possible; however, nobody remains in Cambridge, during my absence, who can send you the strains. I shall be there only at the end of September, so if you are interested to have it before then, please write to the National Collection of Type Cultures, The Lister Institute, Elstree, (England); they should send you the strain in a few days, charging 2s 6d. The No. 123 is the number of the strain in the collection, and the strain is labelled Esch. coli var. acidi Lactici. The Collection may have changed its address recently, but they would forward your letter in this case. I have had troubles to assess the biochemical requirements of the strain. It needs probably cystine or methionine, but results were not reproducible with different samples.

I hope to hear soon from you of new results. My best italian address, in view of the fact that I shall be moving about a while, is the one given below. Wishing you a good work

Yours

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P.S. I am sorry about the insufficient quotation with reference to Prof; Tatum's work. I have not yet had the proofs of the letter to Nature, so I am in time to correct it